Pacific Science (1994), vol. 48, no. 3: 215–218

Results of the 1991 United States–Israel Workshop, "Nutrient Limitation in the Symbiotic Association between Zooxanthellae and Reef-building Corals"[1]

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ABSTRACT: An intensive research effort was organized as a 1-week workshop with the specific goal of resolving difficult questions concerning whether or not zooxanthellae are nutrient limited within host corals. Over 30 scientists participated. Participants applied various techniques to the same set of corals, which had been preincubated under different nutrient regimes for various time periods. Interdisciplinary research projects of this scale and intensity have rarely been attempted by coral reef biologists, yet the evidence developed during the workshop demonstrates the usefulness of this approach. This was a true "workshop" rather than a "talk-shop" and produced important research results while allowing participants to compare research methods and discuss various theories and research philosophies. Important new data were developed. Apparent contradictions were resolved through development of models that consider the dynamics of carbon fixation relative to nutrient availability.

REEF CORALS ARE mutualistic associations between marine invertebrate hosts and microalgal symbionts known as zooxanthellae. Reef corals typically flourish in nutrient-poor tropical and subtropical waters, creating massive biogenic structures. It has been argued that the plant and animal partners in such symbioses exchange vital nutrients and thus have a natural advantage in nutrient-poor tropical waters (Muscatine and Porter 1977). Carbon fixed by algal photosynthesis is translocated to the coral host and inorganic plant nutrients from the animal are taken up by the algae.

Corals are capable of taking up, retaining, and recycling both inorganic and organic dissolved nutrients (Muscatine and Porter 1977, Rahav et al. 1989). In addition, corals obtain nutrients derived from digestion of prey (Erez 1990). The coral *Stylophora pistillata* Esper from the Red Sea, Israel, responds to enrichment with ammonium, or ammonium + phosphate, mostly by increasing the algal density (Muscatine et al. 1989). The same was found for the coral *Pocillopora damicornis* (Linnaeus) from Kaneohe Bay, Hawaii (Stambler et al. 1991). The photosynthetic rate of the nitrogen-enriched colonies increases in comparison with that of controls, although the photosynthetic rate per algal cell decreases (Dubinsky et al. 1990).

There is some question as to whether or not the zooxanthellae are limited by nitrogen or phosphorous while in hospice. Results of previous studies are contradictory. On one hand, the areal density of zooxanthellae in hospice is reported to increase in response to nutrient enrichment (Høegh-Guldberg and Smith 1989, Muscatine et al. 1989, Dubinsky et al. 1990, Stambler et al. 1991). Such increased algal density in response to increased nutrient is the classical response of nutrient-limited populations. On the other hand, flux of nitrogen from host metabolism appears to exceed requirements of zooxanthellae for

[1] Financial support was provided by the U.S.–Israel Binational Science Foundation; University of Hawaii, School of Ocean and Earth Science and Technology; Hawaii Institute for Marine Biology; University of Hawaii Sea Grant Program; and the Edwin H. Pauley Foundation. Manuscript accepted 15 August 1993.

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growth, so the algae should not be nutrient limited in hospice (Rahav et al. 1989). The conclusion that zooxanthellae are nutrient limited as deduced from the increase in zooxanthellae densities in response to nutrient loading is not consistent with conclusions reached by Miller and Yellowlees (1989), based on biochemical indicators. Cook and D'Elia (1987) investigated nitrogen uptake using the nonmetabolizable ammonium analog ^{14}C methylammonium and concluded that zooxanthellae are, in fact, nutrient limited.

Such conflicting results were presented and discussed during the session on nutrient limitation during the Sixth International Coral Reef Symposium in Townsville, Australia, in 1988. These discrepancies may well be due to differences in geographic location, season, cnidarian and algal species involved, or experimental technique. It was obvious that this issue could not be resolved without further investigation, because existing results were based on experiments done in various seas and seasons, under different nutrient levels, and with different organisms. It was agreed that a "hands-on" workshop would be undertaken where participants from different disciplines could work together on the same organisms and samples. The ideal location for such an experiment was the Hawaii Institute of Marine Biology (HIMB) at Coconut Island with its excellent laboratories, access to reefs, and living accommodations.

Participants of the symposium asked P. L. Jokiel and Z. Dubinsky to organize the project. The workshop was sponsored by HIMB in collaboration with the United States–Israel Binational Science Foundation and the University of Hawaii Sea Grant Program and took place at HIMB on Coconut Island, in Kaneohe Bay, Oahu. Over 30 coral reef specialists from the United States, Israel, and four additional countries, among them coral reef biologists, geochemists, physiologists, biochemists, biophysicists, and molecular biologists, joined forces to further our understanding of the nutrient status and fluxes in corals and coral reefs. They brought their various techniques and specialized instrumentation to Coconut Island, and all worked simultaneously on samples from coral colonies that were preincubated for periods of time extending up to 8 weeks under four different ammonium concentrations—nutrient-stripped water ($\ll 1$ μM ammonium), seawater at ambient nutrient concentration (< 1 μM ammonium), and two levels of ammonium enrichment (20 μM and 50 μM). The experiment is described in detail in the following article by Stambler et al. (1994*b*).

The true "experiment" here was not that of nutrient limitation, but rather whether or not such a massive undertaking could be completed within 1 week. This was a true workshop and not a "talk-shop" in that all scientists participated directly in the experiment and worked around the clock. The only organized oral presentations were made during an introductory organizational meeting on Sunday and a summary discussion at the end of the session on the following Friday morning.

RESULTS AND DISCUSSION

Detailed biochemical, physiological, and morphometric data were gathered during the experiment. Results of many of the studies conducted during the workshop are contained in this issue of *Pacific Science*, and other results are forthcoming. A brief summary of detailed findings is as follows:

The carbohydrate, lipid, and protein composition of the zooxanthellae and animal fractions of corals grown under different nutrient regimes were measured (Achituv et al. 1994). Total amino-N and glutamine (gln) to glutamate (glu) ratios were also measured (McAuley 1994). The gln : glu ratios appear to be a sensitive indicator of zooxanthellae response to exogenous nitrogen and suggest that the added ammonium was directly utilized by the symbiotic zooxanthellae. Most of the nitrogen from the elevated seawater ammonium was retained by the zooxanthellae rather than the animal fraction (Muller-Parker et al. 1994*a*), although both plant and animal biomass increased in the 20-μM treatment (Muller-Parker et al. 1994*b*). Analysis of enzymes in the plant and animal fractions

revealed that the animal fraction may play an important role in nitrogen uptake (Yellowlees et al. 1994).

The 50-μM ammonium treatment may have been slightly toxic to the corals. Biomass parameters did not increase with time in that treatment (Muller-Parker et al. 1994*b*). Zooxanthellae in corals exposed to the 20-μM ammonium treatment had mitotic indices (percentage of cells dividing) that were two to three times higher than those of the controls (Høegh-Guldberg 1994). Cells in both ammonium-enriched treatments divided at a higher rate than in the controls. Division of zooxanthellae was still phased in the 20-μM treatment, but there were more cells dividing out of phase compared with the control treatment. Cells in the 50-μM ammonium treatment were out of phase, suggesting destabilization of the symbiosis. Micromorphometric technique (Berner and Izhaki 1994) as well as elemental analysis (Muller-Parker et al. 1994*a*) showed decreasing availability of surplus carbon, giving further evidence of an impaired symbiotic relationship in ammonium-enriched treatments.

The work of Atkinson et al. (1994) emphasizes that delivery rate of ammonium to the coral is controlled by water motion as well as by ammonium concentration. To a degree, "nutrient limitation" must consider "water motion limitation" that involves physical barriers to mass transfer. The results of Muscatine and Kaplan (1994) remind us that corals may become "nutrient-limited" only under high light conditions that allow extremely high photosynthetic rates. Further, different species of corals show different responses to the same alterations in nutrient regime (Stambler et al. 1994*a*).

By the end of the workshop, several of the participants had formulated a model that resolved many previous contradictions (Falkowski et al. 1993). In essence, the argument is made that under normal conditions in nutrient-poor tropical seas, zooxanthellate corals are successful because they are closed systems with respect to nitrogen. Growth of zooxanthellae under these conditions is not balanced with respect to fixed carbon because of the low rate of nitrogen supply. As a result, the excess carbon is translocated to the animal host. Increasing the nitrogen supply leads to rapid growth of the zooxanthellae, with consequent reduction of translocated carbon to the host. Eutrophic conditions allow the zooxanthellae to outgrow their hosts and the host loses control over the population of its symbiotic algae. Thus, maintenance of a balanced coral symbiotic association appears to require low ambient nutrient concentrations. Other aspects of the dynamic carbon-nitrogen model can be clarified by examining the ratio of energy and nutrient fluxes in the regulation of the symbiosis (Dubinsky and Jokiel 1994).

ACKNOWLEDGMENTS

We wish to thank the U.S.–Israel Binational Science Foundation; University of Hawaii, School of Ocean and Earth Science and Technology; Hawaii Institute for Marine Biology; University of Hawaii Sea Grant Program; and the Edwin W. Pauley Foundation for contributing financial support to this project. Living accommodations on Coconut Island for the participants were graciously provided by Mr. Katsuhiro Kawaguchi and the Hachidai Corporation.

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